CHAPTER II

EXPERIMENTAL

1. Materials

The following substances were obtained from commercial sources.

- Tapioca starch (Thai-Wha Co., Ltd., Bangkok,Thailand)
- Potato starch (supplied by Winner Group Enterprise, Bangkok, Thailand)
- Rice starch (m&k , Thai Better Foods Co., Ltd., Bangkok, Thailand)
- Glutinous rice starch (Erawan brand, Cho Heng Rice Vermicelli Fac., Co., Ltd., Bangkok, Thailand)
- Corn starch (supplied by Pharmaceutical Sciences Ltd., Bangkok, Thailand)
- Chloroacetic acid (Lot number 62H0611, Sigma, USA)
- Paracetamol powder USP XXII (Lot number PA 15/224 supplied by Srichand United Dispensary Co.,Ltd., Thailand)
- Methanol anhydrous (Mallinckrodt, USA)
- Absolute ethanol (E. Merck, Germany)
- Isopropanol (Carlo Erba, Germany)
- Glacial acetic acid (E. Merck, Germany)
- Acetic acid (Riedel-de Haen, Germany)
- Sodium hydroxide (Eka Nobel, Sweden)

2. Equipment

- Brabender Amylograph (Model PT 100 , DISBURG, Germany)
- Planetary mixer (Model AR400, Erweka, Germany)
- Oscillating granulator (Kan Seng Lee Ltd., Partnership, Bangkok)
- FGS wet granulator (Model AR400, Erweka, German
- Drum hoop mixer (Model AR400, Erweka, Germany)
- Hot air oven (Memmertt, Germany)
- Magnetic stirrer (model SP-18420, Nuova 7 stir plate, Syborn Thermolyne, USA)
- Analytical balance (Satorious, Germany)
- Moisture determination balance (OHAUS, DISBURG, Germany)
- Carver press
- Hardness tester (Schleuniger, Switzerland)
- Micrometer (Teclock Corp., Japan)
- Disintegration apparatus (Hanson-Research, USA)
- Mechanical sieve shaker (Josef Deckelmann, Aschaffenburg, Western Germany)
- Dissolution apparatus (Hanson-Research, USA)
- Fourier Transform Infrared Spectrometer (model 1760x, Perkin Elmer, USA)
- Scanning Electron Microscope (model JSM-T 220A, Jeol, Japan)
- Roche Friabilator (Erweka, Germany)
- UV/visible spectrophotometer (Milton Roy Spectronic 3000 Array , England)

3. Methods

The experiment was divided into four parts. The first was a preparation of water-soluble of carboxymethyl-ethers of starches. The second was an evaluation of the properties of the modified starches. The third was a preparation of paracetamol granules and tablets. The last was an evaluation of their physical properties.

3.1. Preparing water-soluble of carboxymethyl-ethers of starches

Five native starches used in this study were rice starch, glutinous rice starch, corn starch, tapioca starch and potato starch. These native starches were substituted by three different methods and the methods modified from those described by Filbert (1952).

Method 1

204 parts of anhydrous methanol and 27.6 parts of monochloroacetic acid were throughly mixed and heated to 60 °C. 109 parts of finely divided starch (moisture content 13%) were added with continuous mixing. After that, 55 parts of 50% aqueous sodium hydroxide were added. The reaction mixture was held at 60 °C for 1 hour with good agitation, then cooled to about 40 °C, and then neutralized with acetic acid. After the removal of the mother liquor, the product was washed several times with 80% methanol and finally with anhydrous methanol. The product was dried in a hot air oven at 50 °C over night. The dry product was sieved through No. 80 mesh screen.

Method 2

875 parts of isopropanol and 59 parts of monochloroacetic acid were mixed and heated to 45 °C . 231 parts of finely divided starch (moisture content 13%) were added with agitation, followed by 238 parts of a 50% aqueous solution of sodium hydroxide. After refluxing at about 75 °C for 30 minutes with agitation, the slurry was cooled to about 40 °C, and neutralized with glacial acetic acid. After this, followed the same procedures as mentioned in the method 1, beginning with " the product was washed several times with 80% methanol...".

Method 3

286 parts of absolute ethanol, 29.2 parts of monochloroacetic acid, and 102 parts of starch (moisture content 2%) were slurried, and 38.4 parts of flake sodium hydroxide dissolved in 69.0 parts of water were added with the maintenance of slurry temperature below 50 °C. Agitation at 50 °C was continued for 2 hours. The slurry was cooled to about 40 °C, and neutralized with acetic acid. After the removal of mother liquor, proceeded as directed in the method 1, beginning with " the product was washed several times with 80% methanol...".

3.2. <u>Evalution the properties of modified starches</u>

3.2.1. Determination of degree of substitution

The method to determine a degree of substitution was followed the method as directed for degree of substitution under sodium starch glycolate in USP XXII.

The degree of substitution was obtained by the following procedure.

- Sample 1 gm. (accurate weight) were placed in a 500 ml. glass-stopped conical flask.

- 300 ml. of sodium chloride solution (1 in 10) were added and agitated until starch dissolved.

- Added 25 ml. of 0.1 N sodium hydroxide (Volumetric Solutions ; VS) and inserted stopper.

- The mixture was allowed to stand for 5 minutes with intermittent shaking.

- Then 5 drops of m-cresol purple (Test Solutions ; TS) were mixed.

- After that, 15 ml. of 0.1 N hydrochloric acid (VS) were added, insert stopper and shake.

- If solution is purple, add 0.1 N hydrochloric acid (VS) in 1-ml. portion until the solution was yellow (shaking after each addition).

- Titrated with 0.1 N sodium hydroxide VS until it reached the end point (purple color).

- Calculated the net number of meq (M) of base required for neutralized 1 gm. of carboxymethyl starch, on dry basis.

- Determined the percentage of residue on ignition (C) using sulphuric acid moisture the entire residue after the initial charring step.

- Calculated the degree of acid carboxymethyl substitution (A) by

$$A = \frac{1150\,M}{(7102 - 412M - 80C)}$$

- Calculated the degree of sodium carboxymethyl substitution (S) by

$$S = \frac{(162 + 58A)\,C}{7102 - 80C}$$

- The degree of substitution was the sum of A + S

$$D.S. = A + S$$

3.2.2 Determination the functional group of product

Fourier transform infrared spectrometer (Perkin Elmer, model 1760x, USA) was used to determine the functional groups of the products compared with their native starches.

3.2.3 Moisture determination

The moisture content of the modified starches was determined by using an OHAUS moisture determination balance

(OHAUS DISBURG, Germany). The 3 grams of sample were exposed to an IR lamp set at 1.5 inch mark with an intensity of 6 watt until constant weight was reached. The percent moisture content was calculated from the following equation.

$$\text{percent moisture content} = \frac{(\text{wet mass} - \text{dry mass})}{\text{wet mass}} \times 100$$

3.2.4. Viscosity measurement

This experiment used Brabender[R] Amylograph (model PT100, DUISBURG, Germany), a viscosimeter, which designed to permit a continuous measurement of the viscosity of starch and products containing starch during cooking and, if desired, during cooling with cartridge 700 cmg. to measure the viscosity of native and modified starches by the following method.

Twenty fives grams (except potato starch and glutinous rice starch uesd ten grams), on dry basis, of a starch were suspended in distilled water and made final weight to 500 grams. The suspension was poured into the measuring bowl rotated at 75 rpm. and a measuring feeler with metal pins was projected down into the bowl. The pins provided a good mixture of the sample and prevented the sample from sedimentation. The starch slurry was heated rapidly from 30 to 95 °C at a rate of 1.5 °C/min. It was then held at that temperature for 15 minutes and then cooled to 50 °C. The viscosity values were expressed in Brabender Units (BU).

3.3. Preparation of granules and tablets

The modified starches from 3.1 were used as binders in paracetamol tablets and compared with their native starches and other reference binders. The compositions of paracetamol studied in this study were shown in Tables 1-2 and a batch size for each formulation was 300 gm. The preparation of granules can be divided into 2 methods.

A. Solution incorporation method

Paracetamol was sieved through oscillating granulator (Kan Seng Lee Ltd., Partnership, Bangkok) equipped with a 30 mesh screen. Explotab was incorporated extragranular and intragranular at ratio 1:1. The modified starches, PVP K30 and Era-Gel ^{R} were prepared by slowly dispersed in cold water and adjusted to 8% w/w. The native starches were prepared by heating the starch suspension to be starch paste. All binders were evaluated at 1, 1.5 and 2% dry weight. Paracetamol, lactose and explotab were dry mixed in planetary mixer (Erweka, model AR400, Germany) for 5 minutes. The paste was gradually mixed to the mixture in planetary mixer and kneaded every each portion. After that, further kneading for 5 minutes. Then, the wet mass was screened through an 18 mesh sieve and wet granules were dried in a tray dryer at 50 °C for 6 hours. The dry granules were sieved through a wet granulator (Erweka, model AR400, Germany) equipped with a 20 mesh screen.

Table 1   The compositions of paracetamol tablet formulations in
           solution incorporation method.

Ingredient	Amount (mg./tab)		
	A	B	C
Paracetamol	500	500	500
Lactose	58	55	52
Binder	6	9	12
Explotab	12	12	12
Magnesium stearate	6	6	6
Talc.	18	18	18

Conc. of binder = 8 % w/w
Binder: native starches, modified starches, PVP K30 or Era-Gel[R]

Table 2   The compositions of paracetamol tablet formulations in
           dry incorporation method.

Ingredient	Amount (mg./tab)		
	A	B	C
Paracetamol	500	500	500
Lactose	58	55	52
Binder	6	9	12
Explotab	12	12	12
Magnesium stearate	6	6	6
Talc.	18	18	18
Water (ml.)	0.15	0.15	0.15

Binder: modified starches, PVP K30, Era-Pac[R] or Era-Gel[R]

B. Dry incorporation method

The procedure was followed the same as mentioned in solution incorporation method, except that the binder was incorporated to the mixture in dry form and that the wet granulation was made by transfering water to the mixture.

The granules from the two method were kept in a dessicator for seven days. And then, they were mixed with extragranular disintegrant and lubricant by tumbling for one minute. The blended granules were compressed using 1/2-inch round flat punch and hydraulic press with compression force of 2000 pounds. The weight of each tablet was about 600 mg.

3.4. Evaluation of granule and tablet properties

This part can be subdivided into 3 steps.

1) Evaluated the physical properties of paracetamol granules and tablets containing various types, degrees of substitution and concentrations of the native and modified starches.

2) Chose the formulation from the step 1 which exhibited good physical properties.

3) Compared the formulation obtained from the step 2 with the formulation containing reference binders.

3.1) In solution incorporation method, PVP K30 and Era-Gel^R were used as comparable binder materials.

3.2) In dry incorporation method, PVP K30, Era-Pac^R and Era-Gel^R were used as comparative materials.

The evaluation of granules and tablets was followed under these topics.

3.4.1. Granule evaluations

The following physical properties of granules were examined.

3.4.1.1. Particle size distribution

Particle size distribution of granules was examined by sieve analysis method, using a nest of sieve and a mechanical sieve shaker (Josef Deckelmann, Aschaffenburg, Western Germany). Fifty grams of sample were placed in the top sieve of a US standard sieve series (Endecotts Ltd., London, England) ranging from 850, 425, 250, 180 and 150 um, respectively. Then the nest of sieve was placed on the sieve shaker and shaken for 10 minutes. The results were reported as percentage of weight retained on each sieve size and geometric mean diameter.

3.4.1.2. Bulk density, tapped density and percent compressibility

The bulk density was determined by weighing sample 30 gm. and pouring into a 100 ml. graduated cylinder. Then, the initial volume was recorded and the division of weight by the initial volume showed bulk density. Tapped density was performed by dropping the graduated cylinder on a hard wood surface from a height of 5 cm. until no further reduction in volume was noted. The division of weight by this tightest volume showed tapped density. Both densities were average from three determinations. The percent compressibility was calculated from the following equation.

$$\text{Percent compressibility} = \frac{(\text{T} - \text{B})}{\text{T}} \times 100$$

T and B are tapped and bulk density, respectively.

3.4.1.3. Angle of repose

Angle of repose was determined by funnel method. An amount of 30 gm. of granules was filled in a glass funnel with 6 mm. internal stem diameter fixed on a clamp. The time was recorded when the granule started to flow until finished. Flow rate was calculated in gm./min. and angle of repose was calculated from the following equation.

$$\alpha = \tan^{-1} \frac{H}{R}$$

Where α is the angle of repose, H and R are the height and radius of granule pile, respectively.

3.4.1.4. Percent friability of granule

Ten grams of 20/40 mesh granules and five stainless spheres (each sphere weigh 2.0470 gm. and diameter 7.932 mm.) were filled into a polyvinylchloride container of 9.5 cm. in length and 6 cm. in diameter. The cylinder was fixed in RM 5 Drum hoop mixer (Erweka, model AR400, Germany) rotated at 25 rpm. for 4 minutes. The granules finer than 80 mesh were sieved off by shaking on the sieve shaker for 5 minutes. The granules retained on the 80 mesh sieve (Endecotts Ltd., London, England) were weighed. The percent granule friability was calculated as percentage of weight loss based on the initial weight.

3.4.2. Tablet evalutions

After the granules were compressed to be tablets, the tablets were stood over night before evalutions.

3.4.2.1. Thickness

The thickness of tablet was measured by using micrometer (Teclock Corp., Germany) and expressed in millimeter. The thickness value was an average of ten determinations.

3.4.2.2. Hardness

The hardness of tablet was determined by using the Schleuniger-2E hardness tester (Schleuniger-2E, Germany). The mean and standard deviation of ten determinations were calculated and expressed in kilopound (Kp).

3.4.2.3. Friability

Twenty tablets were dusted, and accurately weighed. Then placed in Roche friabilator (Erweka, Germany) rotated at 25 rpm. for 4 minutes. After that, the fine powder was gently brushed off and reweighed the tablets. Percent friability was calculated from the following equation

$$\text{Percent friability} = \frac{\text{weight loss} \times 100}{\text{initial weight}}$$

3.4.2.4. Disintegration time

The disintegration time of paracetamol tablet was determined in deionized water at 37 ± 2 °C with disk by using a disintegration test apparatus (Hanson Research Corp., Model QC-21, USA) and the procedure was followed according to USP XXII method. The average was calculated from six determinations.

3.4.2.5. Dissolution time

The formulation, using modified starch as a binder and exhibiting the most satisfactory physical property according to 3.4.1-3.4.2, was selected to test for dissolution in comparison with those tablet products using the standard binder.

Nine hundred milliliters of phosphate buffer pH 5.8 ± 0.05 units as the dissolution medium were placed in the glass vessel of the apparatus 2, assembled the apparatus, and equilibrated the dissolution medium to 37 ± 0.5 °C. One tablet was placed in each vessel and the paddle was placed at the center of the vessel and at 2.5 cm. above the bottom of the vessel. The dissolution apparatus (Hanson Research Corp., Model SR-2, USA) was operated at the speed of 50 rpm.

At the time interval of 5, 10, 15, 20, 40 and 60 minutes, five milliliters of specimen were withdrawn from a zone midway between the surface of the dissolution medium and the top of the rotating paddle, not less than 1 cm. from the vessel wall. The same quantity of

medium was added immediately after each sampling to keep the volume of the medium constant during the experiment. After each sample was suitably diluted with dissolution medium, the sample was then determined spectrophotometrically (Milton Roy Spectronic 3000 Array, England) in a 1-cm. cell at 243 nm. and the amount of paracetamol released at any time interval was calculated from standard absorbance-concentration curve presented in Figure 5.

Calibration curve : Paracetamol 250 mg was accurately weighed and dissolved in phosphate buffer pH 5.8 in a 250 ml. volumetric flask. Then, the solution was adjusted with the phosphate buffer to volume. After that, pipetted the solution 1 ml. and diluted with the buffer to 10 ml. and used as stock solution.

The paracetamol dilutions of 2, 4, 6, 8 and 10 ug./ml. were prepared by individually pipetted the stock solution 0.2, 0.4, 0.6, 0.8 and 1.0 ml., respectively, into 10 ml. volumetric flask and adjusted to volume.

The absorbance of each solution was determined by a spectrophotometer (Milton Roy Spectronic 3000 Array, England) in 1-cm. cell at 243 nm. using phosphate buffer pH 5.8 as blank solution. The calibration curve, slope and intercept were determined.

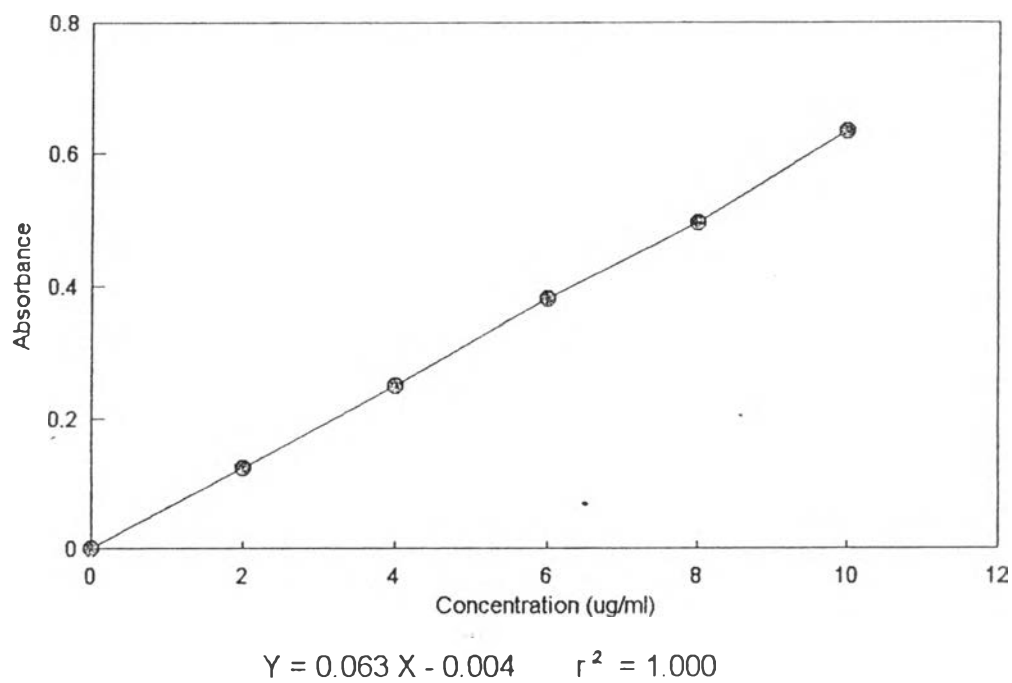$$Y = 0.063 X - 0.004 \qquad r^2 = 1.000$$

Figure 5   Standard curve of paracetamol in phosphate buffer pH 5.8 at 243 nm.